Obesity: **Progress through genetic manipulation**

Allen S. Levine and Charles J. Billington

Transgenic mice have been produced that either lack or overproduce neuroregulatory substances implicated in the control of food intake and body weight. Are such mice useful models for understanding the underlying etiology of obesity in humans?

Address: Minnesota Obesity Center, VA Medical Center and the University of Minnesota, 1 Veterans Drive, Minneapolis, Minnesota 55417, USA.

Current Biology 1998, 8:R251–R252
http://biomednet.com/elecref/09609822008R0251

In recent years, the genes underlying most of the major inherited obesity disorders in the mouse have been cloned, and several transgenic mouse strains have been produced that misexpress regulators of body weight. We classify the genetic manipulations into three groups: those targeting a protein produced in a restricted number of sites outside the central nervous system (CNS); those targeting a broadly expressed regulator of energy metabolism; and those targeting brain neuroregulators and neurotransmitters. We shall try to assess what has been learned from these studies.

Regulators with restricted expression outside the CNS

The crucial role of body-to-brain signaling in weight regulation is evident from the *obese* (*ob*/*ob*) mouse. The gene affected by the *ob* mutation was cloned four years ago, and found to encode a signaling molecule dubbed leptin [1]. Without effective leptin, *ob*/*ob* mice are very obese, infertile and lethargic. Leptin injections, most effectively in the CNS, reverse these abnormalities. Leptin receptors have since been found in key brain sites. It is generally agreed that leptin is a signal necessary for normal body-weight regulation.

At the same time, others have demonstrated the importance of certain peripheral effectors with respect to energy metabolism. Lowell *et al.* [2] found that, in mice expressing a cell-lethal gene from a brown-fat-specific promoter, the lack of brown fat caused serious obesity that included hyperphagia. But knocking out the gene encoding 'uncoupling protein', thought to be the key thermogenic protein in brown adipose tissue, did not produce obesity. These observations were helpful in prompting the successful search for other uncoupling proteins, now known to be expressed in brown fat as well as other tissues.

The enhancer/promoter region of the *aP2* gene, which encodes the adipocyte lipid-binding protein, has been used to direct expression of a specific cDNA to adipose tissue, which has allowed greater knowledge of fat cell function. Such a method has, for example, been used to produce mice that overexpress the beta 1-adrenergic receptor in adipose tissue [3]. These transgenic mice are somewhat resistant to diet-induced obesity, and have smaller adipose tissue deposits. Others have produced mice that overexpress glucose transporter-4 in white and brown fat [4]; they showed a 2–3-fold increase in total body lipid, an increase in fat cell number and obesity. Overexpression of uncoupling protein in white and brown fat prevented the development of obesity in the *yellow* (A^y) mutant mouse (see below) [5].

Widely expressed regulators of energy metabolism

Genetic manipulation can identify previously unknown regulators of energy balance. For example, knockout mice deficient in intercellular adhesion molecule-1 (ICAM-1) became obese in old age, despite a normal feeding regime [6]. When fed a high-fat diet, these mice became obese at a young age. Knockout mice deficient in the counter-receptor for ICAM-1, the leukocyte integrin αMβ2 (Mac-1), also became obese, despite eating similar quantities of food to wild-type mice, suggesting that leukocyte function might influence energy expenditure.

Another example involves basic-helix–loop–helix proteins [7], which include transcription factors involved in growth and development. Loss of such a transcription factor, Nhlh2, made in the ventro-medial and lateral hypothalamus, has sex-specific effects: male mutants are hypogonadal and infertile, and lack instinctive male sexual behavior; female mutants are also hypogonadal, but when housed with males develop normal ovaries and uteri. Both male and female mutants display adult-onset obesity, implicating Nhlh2 in some aspect of the regulation of energy metabolism. A last example concerns cyclic AMP, long known to have effects on cellular metabolism mediated by protein kinase A (PKA), which has two regulatory and two catalytic subunits. Knocking out the gene for the RII regulatory subunit of PKA creates a mouse with very little white adipose tissue, even though food intake is normal [8].

Brain neuromodulators

The experience with genetic manipulation of brain regulators has been illuminating in some cases, but in others the results have been difficult to interpret. One mutation that gives a relatively clear picture is the *diabetic* (*db*/*db*) mouse, which is diabetic and infertile as well as obese, all of which are caused by a splicing defect resulting in an ineffective leptin receptor [9]. This observation underlines the importance of an effective leptin signal from

adipose tissue to the brain, as the most important site of leptin receptors action appears to be in the hypothalamus.

Results that appear to permit straightforward interpretation have also been obtained for the melanocortin receptor system. The *yellow* mouse overexpresses the Agouti protein, which competes with melanocyte-stimulating hormone for binding to melanocortin-4 receptors, resulting in obesity. Knocking-out the melanocortin-4 receptor gene produced mice that developed a maturity-onset obesity syndrome resembling that associated with Agouti overproduction, including hyperphagia, hyperinsulinemia and hyperglycemia [10]. Somewhat similar observations have recently been reported for one type of bombesin receptor. Bombesin is a peptide isolated from frog skin that was found to decrease food intake in several species. Mammalian genes for bombesin-like peptides, which bind to G-protein-coupled receptors, have been cloned. Ohki-Hamazaki *et al.* [11] have found that knockout mice lacking the bombesin receptor subtype-3 become mildly obese, are hypertensive and have impaired glucose metabolism.

In each of the three cases described above, the results can be interpreted as indicating that the particular receptor has a significant role in the regulation of energy metabolism. Interpretation is less straightforward in the case of neuropeptide Y (NPY). Injection of NPY into the hypothalamic paraventricular nucleus dramatically increases food intake, decreases thermogenesis and increases white fat lipoprotein lipase activity and gene expression [12]; chronic injection of NPY produces significant obesity. NPY gene expression increases in conditions associated with an energy deficit, such as food deprivation, high activity, diabetes or lactation. But NPY knockout mice show normal food intake and body weight, and respond appropriately to food deprivation and leptin administration [13]. There is no agreed explanation that reconciles these observations, though one likely factor is that energy metabolism is regulated by redundant mechanisms that can substitute for NPY when it is absent during development. Support for this position comes from the phenotype of *ob/ob* mutants that also lack NPY, which show half the obesity of the *ob/ob* strain [14].

Another complex example is the dopamine-deficient mouse, generated by knocking out the tyrosine hydroxylase gene [15]. Dopamine-deficient mice become hypoactive and stop eating within a few weeks after birth. Within minutes of being injected with L-dihydroxyphenylalanine (L-DOPA), they become active and eat more food than control mice. In this case, specificity is an important issue, as dopamine is known to serve many functions besides the regulation of food intake, and general activity and motor functions may be significantly impaired in the mutant mice.

Studies of the CNS indicate that body-weight regulation occurs through a complex network involving many brain nuclei. Modifying the synthesis of neuroregulators at all sites and developmental times is a relatively crude way of investigating their function, which can give an over-simple picture of the relevant regulatory pathways. While knockout mice models are useful, the lack of any obvious effect on food intake or body weight of a targeted mutation does not mean that the affected gene product can be ruled out as being involved in the regulation of energy metabolism. If one regulator is missing, others can become more salient, and new regulators are constantly being identified. For example, two novel hypothalamic neuropeptides that stimulate feeding, orexin-A and orexin-B, have recently been discovered [16]. Production of these peptides is enhanced in food-deprived rats, as has been noted for other neuropeptides involved in energy metabolism, such as NPY. The effects of modulating gene expression at specific sites and time should lead to a better understanding of the part the gene product plays in body-weight management, and undoubtedly such technologies will be available soon.

References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994, 372:425-432.
2. Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS: Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 1993, 366:740-742.
3. Soloveva V, Graves RA, Rasenick MM, Spiegelman BM, Ross SR: Transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue are resistant to obesity. *Mol Endocrinol* 1997, 11:27-38.
4. Gnudi L, Tozzo E, Shepherd PR, Bliss JL, Kahn BB: High level overexpression of glucose transporter-4 driven by an adipose-specific promoter is maintained in transgenic mice on a high fat diet, but does not prevent impaired glucose tolerance. *Endocrinology* 1995, 136:995-1002.
5. Kopecky J, Hodny Z, Rossmeisl M, Syrovy I, Kozak LP: Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. *Am J Physiol* 1996, 270:768-775.
6. Dong ZM, Gutierrez-Ramos JC, Coxon A, Mayadas TN, Wagner DD: A new class of obesity genes encodes leukocyte adhesion receptors. *Proc Natl Acad Sci USA* 1997, 94:7526-7530.
7. Good DJ, Porter FD, Mahon KA, Parlow AF, Westphal H, Kirsch IR: Hypogonadism and obesity in mice with a targeted deletion of the Nhlh2 gene. *Nat Genet* 1997, 15:397-401.
8. Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, McKnight GS: Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature* 1996, 382:622-6.
9. York DA, Hansen B: Animal models of obesity. In *Handbook of Obesity.* Edited by Bray GA, Bouchard C, James WPT. New York: Deckker; 1998:191-221.
10. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, *et al.*: Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997, 88:131-141.
11. Ohki-Hamazaki H, Watase K, Yamamoto K, Ogura H, Yamano M, Yamada K, Maeno H, Imaki J, Kikuyama S, Wada E, Wada K: Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 1997, 390:165-169.
12. Billington CJ, Levine AS: Appetite regulation: shedding new light on obesity. *Curr Biol* 1996, 6:920-923.
13. Erickson JC, Clegg KE, Palmiter RD: Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 1996, 381:415-421.
14. Erickson JC, Hollopeter G, Palmiter RD: Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* 1996, 274:1704-1707.
15. Zhou QY, Palmiter RD: Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell* 1995, 83:1197-1209.
16. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S: Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998, 92:573-585.